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Sex determination of belugas and narwhals: understanding implications of harvest sex ratio

Détermination du sexe des bélugas et des narvals : comprendre les implications du sex-ratio des captures

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ABSTRACT

Monodontids, narwhal (*Monodon monoceros*) and beluga (*Delphinapterus leucas*), are harvested in the Arctic as part of a subsistence fishery. Maintaining sustainable harvest requires monitoring hunter statistics such as the sex ratio of the harvest. Hunter selection is important harvest information allowing managers to optimize hunter effort (pressure) away from vulnerable groups (i.e., mature females). The sex ratio of harvested animals was determined in the field by inspection of carcasses by hunters and by researchers in the laboratory using genetic techniques. We calculated the technical error rate for narwhal (1.5%) and beluga (7.0%) by comparing reproductive organs to molecular sex determination results. We then determined the field error rate, as the proportional difference between the reported sex and the molecular sex for narwhal (6.3%) and beluga (17.2%). The higher field error rate for beluga is likely due to the lack of distinguishing secondary sexual characteristics. The occurrence of tusked female narwhal in the harvest was also estimated at 6%. To use the precautionary approach of managing stocks or populations increased effort is required to obtain reliable sex ratio determinations for the natural and harvested population. Molecular sex determination can reliably fill this role using available and future hunter collections.

RÉSUMÉ

Les monodontidés, le narval (*Monodon monoceros*) et le béluga (*Delphinapterus leucas*) font l'objet d'une récolte de subsistance dans l'Arctique. Le maintien d'une récolte durable nécessite un suivi des statistiques des chasseurs, notamment du sex-ratio de la récolte. La sélection des chasseurs constitue une information importante sur la récolte et permet aux gestionnaires de concentrer l'effort de la chasse (pression) sur les groupes autres que ceux qui sont vulnérables (soit les femelles adultes). Le sex-ratio des animaux capturés a été déterminé sur le terrain grâce à une inspection des carcasses par les chasseurs et à l'utilisation de techniques génétiques en laboratoire par les chercheurs. Nous avons calculé le taux d'erreurs techniques pour le narval (1,5 %) et le béluga (7,0 %) en comparant les organes reproducteurs aux résultats issus d'une méthode moléculaire de détermination du sexe. Nous avons ensuite mesuré le taux d'erreurs sur le terrain, correspondant à la différence proportionnelle entre le sexe rapporté et le sexe moléculaire pour le narval (6,3 %) et le béluga (17,2 %). Le taux d'erreurs le plus élevé concernant le béluga est sans doute attribuable au fait d'avoir mal distingué les caractéristiques sexuelles secondaires. L'occurrence du narval femelle muni d'une défense dans la chasse a également été estimée à 6 %. L'utilisation de l'approche préventive pour la gestion des stocks ou des populations requiert une hausse de l'effort pour obtenir des résultats fiables concernant le sex-ratio de la population naturelle et de celle chassée. La détermination du sexe par une méthode moléculaire peut jouer ce rôle en recourant aux prélèvements disponibles et futurs des chasseurs.

INTRODUCTION

Models used in the management of wild populations can be improved by using data on the sex ratio of the population and harvest (Legendre et al. 1999). For most vertebrates, harvest rates can be increased and remain sustainable if harvests are preferentially directed towards a specific segment of the population such as males instead of females (e.g., Taylor et al. 1987). For example, harvest models currently used for polar bears (*Ursus maritimus*) include a factor for the proportion of females taken (e.g., Taylor et al. 1987). However, consequences related to size and/or sex selective harvests can arise (McLoughlin et al. 2005, Milner et al. 2007, Allendorf et al. 2008, Fenberg and Roy 2008). Sex selective harvests can have detrimental population level consequences as they tend to reduce the number of sexually mature males in the population. For example, if large males play a disproportionate role in the species ecology (i.e., group defence in primates Wrangham (1986)). In addition, evolutionary consequences can occur after sex selective harvests; for example, trophy hunting has focused on large male bighorn sheep (*Ovis canadensis*) which has caused significant declines in male body mass and horn size (Coltman et al. 2003). This selection has led to a reduction in the number of rams carrying the traits of value to hunters (i.e., weight and horn size) but also the traits that conferred a selective advantage in the first place. Therefore the accurate identification of sex in harvested species is critical for effective management.

Accurate sex determination can often be done by visual examination of external genitalia or secondary sexual characteristics (i.e., antlers in most cervids, plumage in birds) in harvested species. However, in many species the sex of the individual is difficult to ascertain. In cetaceans, genitalia are enclosed with a genital slit and sex is often difficult to determine from a visual inspection; although some exceptions do occur. For example, in northern bottlenose whales (*Hyperoodon ampullatus*) melon shape and color differ between sexes (Jefferson et al. 1993). In many cetaceans, males have characteristics that can be used to distinguish their sex. For example, adult male killer whales (*Orcinus orca*) develop a significantly larger dorsal fin than sub-adults and adult females. Other cetaceans have varying degrees of sexual dimorphism (e.g., size and head shape in sperm whales (*Physeter macrocephalus*)). Of interest here is sex determination in monodontids; narwhal (*Monodon monoceros*) and beluga (*Delphinapterus leucas*). Beluga whales have no distinguishing secondary sexual characteristics although the largest animals tend to be mature males and are reported to become more yellow with age (Huntington 1999). Narwhals are sexually dimorphic in that males develop a large tusk that protrudes from the upper lip to a length of 2 m (Reeves and Tracey 1980). However, a certain percentage of females can develop a tusk (Reeves and Tracey 1980), which seems to vary among harvest locations. Roberge and Dunn (1990) observed 15% (3 of 20) and 5% (1 of 20) of females with a tusk out of all harvested females in Arctic Bay, NU and Pond Inlet, NU, respectively. Hay (1984) estimated the percent of females with a tusk to be between 2.65 and 3.53%. Hay (1984) also estimated that 2.47% of males can reach adult size without growing a tusk.

Given the often cryptic (to humans) nature of an animal's sex, several methods have been developed to obtain these data using genetic methods (summarized in Morin et al. 2005). In mammals, these methods use polymerase chain reaction (PCR) to amplify sex-specific regions on the X and Y chromosomes. A variety of methods and primers have been developed that are 'universal' for mammals (e.g., Shaw et al. 2003) or that are more specific to cetaceans (Palsbøll et al. 1992, Bérubé and Palsbøll 1996, Rosel 2003). This study used the method of Shaw et al. (2003), in which a single primer pair amplifies fragments of an intron within the zinc finger domain. Amplification of male samples will result in two DNA fragments of differing size, one from each of the X and Y chromosomes, whereas female samples amplify two fragments that

are of the same size, one from each X chromosome. As with all molecular methods, there may be some level of error associated with molecular sex determination. This is often due to a low template concentration, which causes the amplification of only a single homologue (Rosel 2003). When this occurs in a male sample, a single band could be interpreted as female.

The objectives of this research are twofold: First, to use reproductive organs and molecular methods to determine the error rate associated with molecular sex determination reaction using the Shaw et al. (2003) primers in monodontids. The focus is on this primer set because it is used in our genetics laboratory (Freshwater Institute, Winnipeg, Manitoba, Canada). Second, to use the molecular sex and reported sex of an individual to determine the error rate associated with sex reported by harvesters and field researchers. These results will help refine the management of these species when using sex ratios reported by harvesters and increase the reliability of molecular sex determination in the laboratory.

METHODS

SAMPLE SOURCES

Beluga and narwhal are important components of the subsistence harvest for northern communities in the Canadian and Greenland Arctic. Fisheries and Oceans Canada is involved in the co-management of these species; as part of this management program whale sampling kits have been distributed to hunters in various northern communities. These kits ask hunters to collect a number of samples and measurements, and also collect data regarding sex, and harvest location and date. This sampling program was expanded in some years to obtain reproductive organs of harvested animals. Samples were frozen in the field and shipped to Fisheries and Oceans Canada, Freshwater Institute, Winnipeg, Manitoba.

MORPHOLOGICAL SEX DETERMINATION

The 'true' sex of each sample was determined by the presence of a uterus and/or ovaries in females and by the presence of testes and/or a penis in males. Reproductive organs were collected by hunters or by researchers in the field at the time of harvest. The entirety of the reproductive organ collection varied between years depending on the parameters of the research project at the time. Collections ranged from simply gonads (testes and ovaries), to a complete reproductive system. Sampling kits were frozen before being returned to the Freshwater Institute in Winnipeg, MB. Reproductive organs were thawed prior to being weighed and measured. Ovaries were fixed in formalin following measurement. Other reproductive organs were destroyed. Some sample kits contained non-reproductive tissues or partial-unidentifiable parts and were omitted.

MOLECULAR SEX DETERMINATION

Genetic samples were preserved in a saturated $NaCl_2$ solution containing 20% DMSO until DNA extraction. DNA was extracted using a variety of methods over the sample collection years from phenol: chloroform to DNeasy spin columns (Qiagen Inc. Valencia, CA) to magnetic bead extraction using the Biosprint 96 platform (Qiagen Inc. Valencia, CA). For kit based technologies the manufacturers protocols were followed with minor modifications to the sample preparation steps aimed at increasing yields from skin tissue samples, which tend to be more difficult to extract DNA from.

Molecular sex was determined using primers developed by Shaw et al. (2003): LGL331 (CAA ATC ATG CAA GGA TAG AC) and LGL335 (AGA CCT GAT TCC AGA CAG TAC CA). Reactions were carried out in a total volume of 10 µl with the reagent concentrations: 1x PCR buffer (Thermopol: 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$; 10 mM KCl; 2 mM $MgSO_4$; 0.1% Triton X-100, pH 8.8 @ 25°C) (New England Biolabs Inc., Ipswich, MA, USA); 0.5 mM $MgCl_2$; 0.2 mM dNTPs; 10%/V BSA; 0.3 µM each primer; and 0.5 U Taq polymerase (New England Biolabs Inc., Ipswich, MA, USA). Approximately 20 ng of template DNA was added to each reaction. Thermocycling conditions consisted of an initial denaturing step of 4 min at 94°C; followed by 30 cycles, each consisting of 30 s at 94°C, followed by 30 s at 55°C, and then 30 s at 72°C; and a final extension of 3 min at 72°C to complete the amplification. Amplification products were visualized either by UV florescence of Sybr Safe DNA gel stain (Invitrogen Corp. Carlsbad, CA) on a 1.5% agarose gel or using the QIAxcel system (Qiagen Inc., Valencia, CA, USA). All samples were scored by eye.

STATISTICAL ANALYSIS

Error rate due to the molecular sex determination reaction was termed as the technical error rate (TER) and quantified as the number of instances where the molecular sex was inconsistent with the morphological sex determination and expressed as a percent. This error could be due to a number of technical errors including sample mislabeling in the field and laboratory, cross-contamination, or non-amplification of one homologue; as precautions are in place to minimize the former two errors the TER is predicted to be due primarily to the latter. The error rate that quantifies the discrepancy between the reported sex and molecular sex was quantified as the number of instances where these determinations were inconsistent. This was expressed as a percent of the total number of samples where the molecular sex was determined and was termed the field error rate (FER).

Pearson's chi squared tests were conducted to determine if the harvest of narwhal and beluga deviated from a theoretical 1:1 sex ratio. These tests were conducted using the stats package in R (www.r-project.org). These were conducted even though the expected ratio is expected to be biased towards males in the narwhal harvest due to the economic benefit of selling the tusk.

RESULTS

NARWHAL

Reproductive organs from 179 narwhals (36 females and 143 males) were examined to determine sex (table 1). Molecular sex was determined for 136 of these samples with two errors. Forty three samples failed to amplify. The resulting technical error rate (TER) for narwhal was 1.5% (2/136) as failed reactions were not considered errors. This error rate was the result of two samples; re-amplification corrected the inconsistency in one sample. Upon re-amplification the second sample was still inconsistent and re-extraction was not possible because the sample had been disposed of. Field and molecular sex were available for 286 narwhal samples and of these 6.3% (18) were inconsistent (field error rate (FER)). The sex ratio in the narwhal harvest was significantly different from a 1:1 ratio ($x^2 = 20.19$, $P < 0.001$) with a bias towards males.

*Table 1. Summary of narwhal data used to derive error rate estimates (%) for technical error (TER = (2/136)*100 = 1.5%) and field error (FER = (16/219)*100 = 7.3) rates.*

Technical error rate		Reproductive Tract (n=179)	
		Male	Female
Laboratory Sex	Male	105	0
	Female	2	29
Failed Reactions (n (%))		36 (25%)	7 (19%)
	Total	143	36

Field error rate (7.3%)		Laboratory Sex (n=219)	
		Male	Female
Reported Sex	Male	121	9
	Female	7	82

BELUGA

Reproductive organs from 237 belugas (91 females and 146 males) were examined to determine sex (table 2). Molecular sex was determined for 172 samples (65 failed amplifications) and of these, 12 samples did not correspond to the sex determined by examining the reproductive organs. The resulting TER for beluga was 7.0% and of these 12 samples, re-amplification resolved all inconsistencies except for three. Subsequent re-extraction of the three samples was not possible due to these samples being disposed of. Field error rate (FER) was calculated as 17.2% for 688 beluga samples. The sex ratio in the beluga harvest was significantly different from a 1:1 ratio ($x^2 = 28.49$, $P < 0.001$) with a bias towards males.

*Table 2. Summary of beluga data used to derive error rate estimates (%) for technical error (TER = (12/172)*100 = 7.0%) and field error (FER = (118/688)*100 = 17.2%) rates.*

Technical error rate		Reproductive Tract (n=237)	
		Male	Female
Laboratory Sex	Male	85	2
	Female	10	75
Failed Reactions (n (%))		51 (35%)	14 (15%)
	Total	146	91

Field error rate (17.2%)		Laboratory Sex (n=688)	
		Male	Female
Reported Sex	Male	346	68
	Female	50	224

DISCUSSION

Overall, the error rate associated with our molecular sex determination in the lab was low for narwhal (1.5%) and beluga (7.0%). The difference in error rate between the two species may be due to an increased laboratory effort that has been placed on the narwhal dataset. All narwhal samples were re-profiled and many were re-extracted in 2008-2010 using consistent methodology. The beluga samples have been profiled in an ongoing fashion so multiple methodologies have been applied. For example, DNA quantification is currently an integral step

in the current protocol and low concentration samples are identified and re-extracted before sex determination reactions are performed. Alternatively, the sex determination reaction was slightly more efficient in narwhal compared to beluga. However, the rate of failed reactions was similar in both narwhal (24%) and beluga (27%) suggesting that this is not the case. This failure rate may be reduced if the primers were redesigned to amplify a smaller fragment in the same region as has been done for caribou (*Rangifer* spp) (Ball et al. 2007). In general most errors occurred where the molecular sex was determined as female when in reality the sample was male. This error would be expected when template concentrations are low and only one of the homologues is amplified. The post-extraction quantification of DNA is important in this respect to ensure that samples have sufficient high quality DNA to amplify both the X and Y fragments.

With technical error rates established, the error rate associated with field collection is estimated at approximately 6.3% (+/- 1.5% (TER)) for narwhal. The field error rate was much higher for beluga (17.2% +/- 7.0%) and is likely related to the lack of secondary sexual characteristics that can be used to infer the sex of the animal. Harvested animals may not be dressed out further than flensing because in many communities the meat is no longer used to the degree it was in the past (Roberge and Dunn 1990, Kilabuk 1998). Therefore, hunters may not be examining carcasses in detail but are relying on their knowledge and experience to infer the sex of an individual (i.e., based on size, colouration). As expected if the error in field sex determination was random, the number of female beluga reported as males is approximately equal to the number of males reported as females.

FEMALE TUSKED NARWHAL

The percentage of female narwhal with tusks reported in Roberge and Dunn (1990) lead to the expectation that a high proportion of tusked females would be identified in our sample. However, this was not the case. Examination of the sampling data from 1980 to 2010 (table 3) indicates that most communities have reported harvests of females with tusks, with the overall percentage of tusked animals being reported as female at 6% (calculated using only communities for which data on tusk length was available for more than 30 animals). This rate varied among sampling locations; however, sampling was not random therefore it is not possible to reliably extrapolate these values to the entire population. These results suggest a higher incidence of females with tusks than observed by Hay (1984) who suggested two to three percent based on a sample of 62 netted animals. It is interesting to speculate on why this percentage is higher than observed for other mammals with male secondary sex characteristics. Although usually not rigorously collected, Donaldson and Doutt (1965) collected observations of 165,000 hunted white-tail deer (*Odocoileous virginianus*) where only 17 were functional females. The higher proportion of female narwhal with tusks may support a secondary role for the tusk as suggested by Nweeia et al. (2009).

Table 3. Summary of the sex of tusked narwhal landed in eastern Arctic communities. The sex is reported from sample collection datasheets. Samples are not evenly distributed among years within the range of years presented. Mean and standard deviation (SD) of exposed tusk length is reported in cm, along with the sample size (N). The percent of tusked animals that were female (TF) is reported for communities with greater than 30 animals with tusk data. Sample sizes for each location-category have been indicated in bold.

Location (Range of years)	Summary	Male	Female	Total	%TF
Arctic Bay	**N**	**166**	**11**	**177**	**6%**
(1983 to 2009)	Mean	167	124	165	
	SD	46	42	46	
Qikiqtarjuaq	**N**	**49**	**3**	**52**	**6%**
(1993 to 2008)	Mean	141	120	140	
	SD	54	56	54	
Coral Harbour	**N**	**2**	**3**	**5**	
(1995)	Mean	173	139	152	
	SD	78	82	72	
Clyde River	**N**	**9**	**1**	**10**	
(1993 to 2005)	Mean	117	23	108	
	SD	47	NA	53	
Grise Fiord	**N**	**37**	**2**	**39**	**5%**
(1999 to 2007)	Mean	206	131	202	
	SD	304	102	297	
Igloolik	**N**	**5**	**2**	**7**	
(2006)	Mean	153	111	141	
	SD	27	5	30	
Pangnirtung	**N**	**3**	**1**	**4**	
(1990 to 2010)	Mean	121	20	95	
	SD	107	NA	101	
Pond Inlet	**N**	**126**	**8**	**134**	**6%**
(1982 to 2006)	Mean	133	49	128	
	SD	54	30	56	
Repulse Bay	**N**	**40**	**2**	**42**	**5%**
(1993 to 2009)	Mean	133	69	130	
	SD	49	35	50	
Resolute Bay	**N**	**2**	**1**	**3**	
(2002 to 2009)	Mean	156	53	122	
	SD	5	NA	60	
All Locations with greater than 30 samples	**N**	**418**	**26**	**444**	**6%**
(1982 to 2010)	Mean	156	99	153	
	SD	31	37	31	

MANAGEMENT

In terms of management, the goal is to maintain viable populations and stocks in order to ensure the long term sustainability of the resource. This involves assessing the risk associated with various harvest levels given the current data on abundance, population growth, and harvest levels. The risk related to not accounting for the error in the estimated sex ratio of the harvested animals is unknown. However, it could be significant. It is important to continue to assess the

sex ratio of the harvest and attempt to determine the natural sex ratio of narwhal populations. Narwhal have many characteristics in common with other large mammals that are actively managed. These include: long life spans, slow reproductive rates, and extended maternal care of young (Hay 1984). In these respects narwhal can be compared to polar bears for which Taylor et al. (1987) suggested that the sustainable harvest of females was only 1.0 to 1.6% above the natural mortality. Given the similarities it might be expected that the sustainable harvest of narwhal females would be similarly low. Therefore, a small error in the assessment of the harvest sex ratio could be important for the sustainability of the harvest. Beluga share many life history traits with narwhal and the sex ratio of the harvest will be important to manage. However, it is very difficult (if not logistically impossible) to identify the sex of a beluga before it is harvested and therefore sex ratio management would be difficult.

Currently, market pressures are promoting a male bias in the narwhal harvest from most communities. While this is generally considered preferable in game management, recent research has identified population and evolutionary consequences to an extreme, or sustained, male bias in the harvest. Male-selective harvests have been linked to overall reductions in male characteristics that have been correlated with fitness (Coltman et al. 2003). In addition it has been hypothesized that extreme female bias in a population leads to population level consequences because females cannot find mates (Allee effect, Molnár et al. 2008).

In the future it is likely that monodontid population will be harvested at higher rates and be impacted by numerous other anthropogenic activities including climate change, increased shipping, and fishing in Baffin Bay (Laidre and Heide-Jorgensen 2005, Laidre et al. 2008). If populations and stocks are managed sustainably it will be important to have an accurate assessment of the sex ratio of the harvest. In narwhal, the harvest is monitored through the use of tags that are issued to hunters in each community. Under this system, each landed narwhal is issued a tag that is used to track legitimate exports of narwhal products out of Nunavut and Canada. To improve the management of this species, a responsibility of the hunter should be to include the submission of a tissue sample for genetic analysis and basic morphological data to refine population models. This should also be complimented with research into the natural sex ratio of the population which may naturally deviate from 1:1.

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